THE AERIAL STEM AND LEAF-SCALE ANATOMY
OF MONOTROPA UNIFLORA L.:
A DESCRIPTIVE STUDY

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ABSTRACT

Light microscopic investigation of the aerial stem and leaf-scale anatomy of heterotrophic Monotropa uniflora L. confirmed major clues supportive of a probable photosynthetic ancestor. Observation and measurement of callose plug diameters indicated uniformity in pore size between those found on the lateral cell walls and those located on the end walls. There was evidence for a possible intercellular connection between a sieve tube member and its associated parenchyma cell even though companion cells have not been described for Monotropa uniflora L. by earlier researchers. The presence of sieve tube member nuclei and P-protein were not indicated. These structural features approach those reported earlier for lower vascular plants. Sieve tube member occurrence decreased acropetally culminating in total absence of detectable sieve elements in the leaf-scales. This does not coincide with phloem distributional patterns described for photosynthetic vascular plants. The reduction of sieve element specialization and characteristic distribution were probably brought about by the evolutionary loss of photosynthetic capability.

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INTRODUCTION

The achlorophyllous condition exhibited by many of the flowering plants has long been a subject of interest to botanists. How do these plants compare with photosynthetic angiosperms physiologically, ecologically, and anatomically? What are some of the possible evolutionary tendencies displayed by heterotrophic angiosperms?

The present study was initiated to investigate the anatomical features of the aerial stem and leaf-scale system of *Monotropa uniflora* L. The major question raised from the previous anatomical studies of *Monotropa* asks what affects the loss of the photosynthetic capacity has had on the vascular tissue, especially the phloem. Gleeson (1970) studied the anatomy of *Monotropa* roots and stem, but chose not to elaborate on sieve element cytology. Henderson (1919) conducted a comparative structural study on members of the Pyrolaceae and Monotropaceae. Her description of *Monotropa* leaf-scales was the most recent to date.

This paper will attempt to present new information on the overall stem and leaf-scale anatomy with emphasis on the cytology and distribution of the phloem through light microscopic observations.

LITERATURE REVIEW

An adequate historical review of the classification of Monotropa uniflora L. has been presented by Gleeson (1970). At present, it is accepted by researchers (Henderson, 1919; Mac Dougal and Lloyd, 1900; and Nowicke, 1966) as belonging to the herbaceous family Monotropaceae in the order Ericales. Monotropa uniflora L. appears above ground in June or July and being achlorophyllous in nature exhibits a waxy white color (Fig. 1). The plant stem bears numerous leaf-scales arranged in a regular spiral pattern tightly oppressed to the axis with the leaf-scales grouped closely together at the base and gradually becoming more segregated towards the tip. At the apex, there exists a single white flower which remains in a "nodding" orientation until fruit development. The aerial plant body may appear singly, in pairs, or in a clump of three or more in late August or September. The height may be anywhere from 1-2 decimeters (Britton and Brown, 1963). The aerial portion remains fleshy during youth through early maturity, but in late maturation, the entire above ground structure begins to turn brown-black and soon dries out. The dried stalks remain erect through the winter and sometimes into the next season.

Monotropa uniflora L. contains less than 0.001 mg of chlorophyll per gram fresh weight (Lutz and Sjolund, 1973). The minute amount of chlorophyll and the conspicuous absence of chloroplasts prevent the plant from carrying out the photosynthetic process. The actual mode of nutrition for the plant has been a subject of debate between those researchers who maintain Monotropa is a saprophyte (Brooke, 1938; Chansler, 1950; Clute, 1931; and Hardy, 1917) and those who brand the plant as a parasite (Esau, 1965; Fahn, 1974; and Priest, 1926).

A well developed mycorrhizal habit seemed to be the evolutionary solution to the problem of an organic nutrient source. Gleeson (1970) confirmed the roots as being covered by a dense mat of fungal hyphae. A closer look at the mycorrhiza by Lutz and Sjolund (1973) revealed the roots to exhibit both intercellular and intracellular hyphal infection which made the association a prime example of an ectendomycorrhiza (Hacskaylo, 1972). The infected root cells have pronounced cell wall projections very much like those found in transfer cells and this observation lead Lutz and Sjolund (1973) to conclude that Monotropa uniflora L. was exceptionally well adapted structurally to exploit its fungal associate.

Bjorkman (1960) suggested that there is a third member to the nutrient flow system of Monotropa. The third member proposed was a chlorophyllous vascular plant which shares a common mycorrhizal fungus with the Monotropa. Radioactive glucose (D-glucose-^{14}C) was injected into the secondary phloem of mature pine and spruce trees known to have

Monotropa hypopithys L. growing beneath them and it was found that carbohydrates are in fact translocated from the trees to the Monotropa via the fungus (Bjorkman, 1960). As a result of his experiments, Bjorkman (1960) concluded that Monotropa hypopithys L. is an epiparasite on the roots of photosynthetic vascular plants.

The question arose as to whether or not an exchange of materials takes place between the Monotropa and the associated vascular plant via the common fungus. Furman (1966) tested this possibility by injecting ^{32}P into the stems of Monotropa uniflora L. He found that ^{32}P was translocated from the Monotropa to the photosynthetic vascular partner by way of the fungus. Furman (1966) advocated the possibility that there is a three-way symbiotic relationship between the Monotropa, a vascular plant containing chlorophyll, and a fungus due to the exchange of vital materials in a three-phase mycorrhizal structure. The fungus involved in the symbiosis was identified as Armillaria mella (Campbell, 1971). He found that this basidiomycete formed rhizomorphs with both Monotropa uniflora L. and members of the Acer, Fagus, Pinus, Quercus, and Tsuga genera.

Kuijt (1969) conceived an angiosperm parasite relationship as one involving a haustorium-like structure, through which water and other essential nutrients can be transported from host to parasite. Those who believe Monotropa to be parasitic (Esau, 1965; Fahn, 1974) support their view by the study of Lutz and Sjolund (1973) which showed Monotropa uniflora L. to fit the structural requirements for Kuijt's concept of an angiosperm parasite. Kuijt (1969) argued that a broad notion of parasitism should not be accepted as to include mycorrhizal relationships due to the great numbers of nonparasitic vascular plants which readily form mycorrhizae (Hacskaylo, 1972). Even though Bjorkman (1960) presented evidence for the epiparasitic nature of Monotropa on the roots of chlorophyllous vascular plants, there was evidence to show that substances may be supplied to the associated partners by the Monotropa (Furman, 1966) and may be a true symbiont (Mac Dougal and Lloyd, 1900; Moore, 1929). The experimental evidence to date is insufficient and inconclusive concerning the exact position occupied by Monotropa uniflora L. in the nutritional scheme.

Observers from East Asia, across the breadth of North America, and as far south as Central America have gathered documentation for the occurrence of Monotropa in their areas (Britton and Brown, 1963). Among those who have reported (Brooke, 1938; Campbell, 1971; Chansler, 1950; Clute, 1931; Hardy, 1917; and Priest, 1926), the plant was consistently found in moist humus woods. This type of habitat would provide Monotropa uniflora L. with a ready access to the soil fungus Armillaria mella with which to form the characteristic three-way mycorrhiza (Campbell, 1971). There was some deviation from the humic forest habitat (Hull, 1914).

Monotropa uniflora L. was found growing in the typically xerophytic oak dunes of Lake County, Indiana. He asserted that the plant blooms later in the fall than previously documented for other habitats and to complicate the story further, this seems to be the exact reverse of early flowering initiated by the xerophytic habit. A reasonable explanation seems to be that the plant was capable of adjusting to and surviving under xerophytic conditions due to its short duration above ground (Hull, 1914).

The question concerning the ecological significance of Monotropa and its symbiotic partners was considered by Furman and Trappe (1971). They suggested that the photosynthetic vascular plant served as the producer, the fungus as nutrient gatherer and recycler, and Monotropa as the metabolite producer and temporary storage vessel. Due to the variety of ecosystems, habitats, and organisms involved various researchers (Campbell, 1971; Furman and Trappe, 1971; and Hull, 1914) feel it unwise to formulate and advocate too many generalizations. Even with improved physiological and ecological methods, the many questions raised remain unanswered.

MATERIAL AND METHODS

Approximately 12 aerial specimens of *Monotropa uniflora* L. were collected in La Crosse and Oneida counties in Wisconsin. Three of the plants at various stages of chronological development were collected in October 1974 from a wooded area near Grandad Bluff. A fourth plant was obtained in fruit on September 1, 1975 in the same general area in La Crosse County. Eight other specimens were taken on July 19, 1975 in a heavily wooded area in Oneida County about two miles north of the University of Wisconsin's Kemp Biological Field Station. All specimens were field fixed in FAA (Sass, 1958). The specimens were later cut into sections measuring one centimeter and aspirated in fresh FAA two days after the initial tissue fixation. These tissue samples were used to make serial cross and longitudinal sections and stelar macerations.

Serial Sections

Four entire aerial stems were selected for serial study, each plant representing a different developmental stage ranging from young to the mature. Keeping the four plants separate, each stem section was catalogued as to its relative position in the axis and then dehydrated using an ethanol sequence. After dehydration the stem portions were infiltrated with paraffin, mounted on blocks, and sectioned on a rotary microtome at 8-15 microns. The serial sections were affixed to glass slides and stained with Foster's tannic acid, ferric chloride, and lacmoid (Cheadle, Gifford, and Esau, 1953) to determine the presence of lignin and callose. Cover slips were mounted on the slides with Harleco Synthetic Resin.

Macerations

Four of the remaining aerial stems were prepared for tissue maceration according to Sass (1958). The epidermal and most of the cortical layers were removed leaving only the stele. The stelar portions were then cut longitudinally into thin slivers for better tissue exposure. The splinter-like sections were treated with Jeffrey's Solution (1:1 of 10% nitric acid to 10% chromic acid) for 20 hours at room temperature. The sections were then washed with distilled H_2O and stained with Foster's lacmoid sequence. After the slivers of tissue were destained, the cells were then teased apart with jeweler's forceps. Aggregates of cells were then mounted in a glycerin-ethanol solution for observation.

Epidermal Peels

The last four FAA preserved aerial stem sections were stripped of epidermis and underlying cortex. The cortical cell layers were peeled away from epidermis and discarded while strips of epidermis were placed in Lugol's Solution (I_2KI) for 15 minutes as a contrast stain. The tissue was then rinsed in 70% ethanol and mounted on glass slides in a glycerin-ethanol solution for light microscopic observation.

Quantitative Measurements

Observations were made through a Leitz Dialux light microscope. Cell measurements were made at different magnifications with a calibrated Bausch and Lomb stage micrometer and ocular micrometer. Cell measurements for length were taken between the farthest oblique portions of the end walls and between the outermost point of the lateral walls for diameter measurements.

AERIAL STEM RESULTS

Epidermis

A uniseriate system of elongate cells comprise the epidermis. Individual epidermal cells are maintained by evenly thickened cell walls that make the cells appear irregular in shape. Transverse and longitudinal observations reveal the external tangential wall to consist of a network of well defined ridges. A very large nucleus with several nucleoli dominate the protoplast. Epidermal cells may also collect and apparently store certain materials. Longitudinal and transverse sections display an unidentified substance concentrated into globular vesicles and arranged in a peripheral pattern around the inside of the cells (Fig. 4). The number of cells exhibiting this phenomenon increased in an acropetal direction.

Examination of numerous epidermal peels confirm the presence of stomata (Gleeson, 1970; Henderson, 1919) and the morphological appearance of the guard cells was found to coincide with Henderson's (1919) description (Fig. 7, 8). The guard cells are smaller than the surrounding epidermal cells and either lie parallel to or somewhat perpendicular to the long axis of the stem. No assessment of stomatal frequency was attempted in this study due to the difficulty encountered in locating good demonstrative examples.

Cortex

A multiseriate system of irregularly shaped, polyhedral parenchyma cells characterized the cortex. The depth of the cortex varies with the fleshiness of the individual stems, but was frequently observed to be between 14-25 cell layers thick with intercellular spaces plainly visible (Fig. 2, 3). The size of individual cells increased slightly with increased distance from the epidermis while maintaining the characteristic uniformity in cell wall thickness. The nucleus is large and can be observed to enclose one to several nucleoli.

Foster's lacmoid stain (Cheadle, Gifford, and Esau, 1953) demonstrated that at least two different storage materials were found to occur. One type being similar to the peripheral substance found in the epidermis and the other type taking the form of distinct spherical to ellipsoid bodies. Both of the substances were observed to increase in abundance in the middle and upper portions of the stems.

Cross sectional and longitudinal views of several stem sections from plants collected in both La Crosse and Oneida counties displayed insect infestation. The unidentified immature insect forms appear to be in the developmental process of their species while residing in distinct cortical chambers just under the epidermis (Fig. 5, 6). Large numbers of the insect forms were found to occur within the confines of several individual plants. This observation has never been reported to occur in Monotropa uniflora L.

Stele

The arrangement of the vascular bundles is eustelic (Fig. 2, 3). A lignified parenchyma bundle enclosure varies in width from 1-5 cell layers. This interpretation is in agreement with the assessment made by Gleeson (1970). The individual cells of the stele are elongated and smaller in diameter than the cortical cells.

The medullary pith-ray parenchyma and the larger pith parenchyma of mature basal stem are lignified (Fig. 3). These tissues decrease in lignin deposition with increased distance from the root system. The apparent decrease in lignification is also displayed by the bundle delimiting parenchyma. This fact makes it increasingly difficult to distinguish individual bundles in apical portions of the stem (Fig. 2). The number of bundles varies from approximately 9 at the base, increasing to 18-20 in the middle, and then again decreasing to around 10 at the apex of the plant.

Observation revealed that storage material similar to that found in epidermis and cortex is present in the pith, medullary rays, and bundle parenchyma. The frequency of this material follows the general pattern already established in other tissues with a minimal amount at the base and increased amounts in the middle and upper portions of the stem.

The Vascular Bundle

The general size of each bundle is dependent on the fleshiness of the plant and varies accordingly, but the overall size of the vascular bundles seems to decrease in the direction of the apex with lessening numbers of vascular elements. Individual bundles are delimited by lignified parenchyma cells. The bundle cells can be thought of in terms of three different cell groups as defined by Esau (1965): sieve tube members, parenchyma, and tracheary elements.

Sieve elements as determined by serial section are arranged in clusters of 3-6 cells. Each cluster is surrounded by parenchyma cells exhibiting dense protoplasts with rather large nuclei (Fig. 11, 12). The sieve element clusters and their associated parenchyma cells are positioned in an arch around the tracheary elements. The numbers of tracheary elements may range from 8-40 cells depending upon the size of the bundle. Tracheary elements possess thickened, lignified secondary wall material as determined by Foster's lacmoid. These water conducting elements remain aggregated within the region nearest to the pith, thus establishing a collateral type bundle (Esau, 1965). Present observations are confirmed by the report submitted by Gleeson (1970).

The inner bundle parenchyma appear capable of storage (Fig. 9). A sectional examination demonstrated quite clearly that material of varying morphological appearance collects within the confines of these cells. The amount of this material increases with distance from the roots. No such substances were seen to occur in sieve or tracheary elements proximal to the storage cells.

Tracheary Elements

Microscopic investigation of the xylem substantiated the findings of Gleeson (1970). Protoxylem is composed of tracheids with uniformly deposited primary wall. These cells exhibit characteristic annular, helical, and scalariform type secondary wall deposition (Fig. 10) and range in lengths of 700 microns to 1500 microns maintaining a width approximating up to 15 microns. Metaxylem is characterized by scalariform tracheids and vessel members. Vessel members are classified as such on the basis that they possess scalariform perforation plates on their oblique end walls. The cells can be found arranged in vertical fashion, one on top of the other. Vessel members tend to be shorter than tracheids and measure 400 to 700 microns in length with 18 microns as a commonly measured width.

Xylem differentiation and development are centrifugal with respect to the location of the tissue within the bundle as reported by Gleeson (1970). The secondary thickened wall material can be seen in various stages of lignification due to the lacmoid staining process (Cheadle, Gifford, and Esau, 1953). The lignified secondary wall stains very blue while developing walls still appear brown-black in color. For a more indepth look at xylary element ontogeny, the reader is referred to the anatomical contribution made by Gleeson (1970).

Sieve Tube Members

The employment of Cheadle, Gifford, and Esau's (1953) lacmoid stain for callose enables light microscopy to reveal sieve tube member characteristics. According to Esau (1969), callose is an important aspect in sieve element identification. Sections of phloem studied in both transverse and longitudinal views brought to light several new perspectives on Monotropa uniflora L. structural cytology not previously observed. Terminology used to describe structural characteristics of sieve tube members is taken from Esau (1969). For a visual perspective on the measurement reference points used in describing features, the reader is referred to Fig. 21.

Sieve elements studied appeared with cell lengths ranging from 136 to 278 microns with the majority of those observed falling into the 186 micron category. Sieve element diameters were found to vary between 5.5 and 16.5 microns. These findings concur with those made by Gleeson (1970). The thickness of the lateral cell walls range from 0.55 to 1.1 microns.

Scattered along the lateral walls with their characteristic callose plugs are sieve areas (Fig. 15). The callose plugs denote the presence of sieve pores usually numbering between 1-7 in a single sieve area. The size of the lateral sieve area may vary in diameter and depends upon the number of pores present. In viewing the callose plugs, it was not known how much callose overlap existed. The pores can be no larger than the diameter of the plug and may be smaller. The diameter of the visible callose plugs were 0.55 to 1.1 microns. It is of interest to note that numerous lateral sieve areas were shown to consist of just one or two callose plugs. Sieve areas serve as a means of protoplast connection between two or more sieve elements (Esau, 1969) and are shown in this capacity in longitudinal view (Fig. 16).

The end walls of the sieve tube members are slightly oblique (Fig. 14). Perforating the end walls are numerous callose plugs, again indicative of the existence of pores (Fig. 11, 14). The callose plugs measured were small, approximately 0.55 to 1.1 microns. Although close to the size of the lateral sieve area plugs, the end wall callose plugs are not arranged in any specific pattern and form a simple sieve plate. Sieve plate diameters range in the 7-12 micron category and at no time did the sieve plates indicate the presence of open pores, only the callose plugs were visible. Sieve plates were shown to lie in contact with other sieve plates between vertically aligned sieve elements, thus forming sieve tubes (Fig. 14).

Examination of sieve tube member protoplasts failed to reveal the presence of P-protein or nuclei. The inner wall callose lining stained blue with lacmoid. At no time was there any storage material observed within these cells.

Special Observation

Transverse section through a mature stem vascular bundle exhibited sieve elements and parenchyma in close association. Situated between a nucleated parenchyma cell and an enucleated sieve element can be seen a connecting substance (Fig. 12). This substance appears to have been deposited on both the inner cell wall of the parenchyma cell and on the inner wall of the sieve element. When viewed under the light microscope, this substance was blue in color. Since this section was stained with lacmoid (Cheadle, Gifford, and Esau, 1953), the color and location of this substance is indicative of callose. Certain longitudinal views have also eluded to the possibility of callose detectable connections between sieve tube members and their associated parenchyma (Fig. 16), however, the absence of clear concise views of this phenomenon make positive identification impossible.

Esau (1969) describes companion cells as having a lateral pit area in connection with a lateral sieve area of an adjacent sieve element. Companion cells have never been found to exist in Monotropa uniflora L. (Gleeson, 1970; Henderson, 1919), but this present observation warrants further investigation.

LEAF-SCALE RESULTS

Epidermis

Leaf-scale epidermal cells are similar in structural appearance to those cells which comprise the stem epidermis. The epidermis is formed by a single continuous layer of cells on both the abaxial and adaxial surfaces (Fig. 17). The cell walls appear to be evenly thickened with the outer wall revealing characteristic ridges. Each individual cell has a distinct nucleus with a varying number of nucleoli.

Although stomata have been reported by previous researchers (Gleeson, 1970; Henderson, 1919), surface examination of adaxial and abaxial epidermal sections in the present study did not confirm these earlier observations. Recent inquiry does, however, disclose a possible storage function for the epidermis. The storage material appears brown in color when tissue sections are stained with lacmoid (Cheadle, Gifford, and Esau, 1953). Transverse sections made through epidermis indicated that the unidentified material assumes various morphological patterns or may fill the lumen evenly. The leaf-scales located near the apical portion of the aerial axis showed a greater tendency to accumulate the substance.

Mesophyll

The inner leaf-scale tissue is not differentiated into spongy or pallisade layers which confirms Henderson's (1919) report. The mesophyllous tissue is composed of thin walled parenchyma cells. These cells can be described as polygonal with definite nuclei and nucleoli (Fig. 18, 19). No plastids of any kind were detectable on the light microscopic level. The cells are arranged to fit very tightly with one another leaving no intercellular spaces. No form of storage material was contained within this tissue region.

Venation

Vascularization of the leaf-scales is accomplished by three or more prominent veins running parallel to the longitudinal axis and originating from a single leaf trace (Fig. 20). Branching away from the larger veins, minor veins penetrate the mesophyll with only the parallel venation pattern. The number of veins varies with the size of the individual leaf-scale and the fleshiness of the plant which supports it.

The cellular composition of the veins has two major constituents. The dominant element of the vein is based on helical and reticulate tracheary elements arranged in groups of 2-10 cells. More water conducting elements clustered into bundles make the vein even more prominent. The other element of the vein is vascular bundle parenchyma. These vascular bundle parenchyma cells are smaller in diameter and possess protoplasts that are more dense than the mesophyll parenchyma (Fig. 18, 19). The cells are nucleate with nucleoli and at no time did any sectional view indicate the presence of callose. The absence of callose and detectable sieve areas prevents the classification of these cells as Esau (1969) defined sieve elements. The absence of callose and detectable sieve elements has not been observed in previous studies.

DISCUSSION

Contributions by Henderson (1919), Gleeson (1970), and the present study bring to light various anatomical features which lend support to Copeland's (1941) presumption of an autotrophic ancestor for the heterotrophic *Monotropa uniflora* L. The opaque appearance of the aerial stem, the presence of less than 0.001 mg of chlorophyll per gram fresh weight (Lutz and Sjolund, 1973), and the existence of epidermal stomata constitute the major clues to the plant's ancestral model. The present study verifies Henderson's (1919) report on the occurrance of stomata, but also draws attention to the absence of sub-stomatal chambers as described by Esau (1965). A plant that is incapable of building up and maintaining its own photosynthetic reserve would have very little use for such a chamber to facilitate substantial uptake of CO_2 for photosynthesis. The stomata may play an active role in the gas exchange associated with cellular respiration.

Insight into the evolutionary scheme of the higher plants is best gained through study of the vascular tissue (Esau, 1965; Fahn, 1974). By virtue of their perspective functions, the vascular tissues are arranged in the plant body in the most efficient manner. According to Esau (1965) and Fahn (1974), the xylary elements should be well developed in the region of the roots and evenly distributed throughout the plant delivering water and minerals to the sites of the greatest photosynthetic activity located in the leaves and stem. Esau (1969) also proposes that for photosynthetic vascular plants, the sieve element distributional situation should be the reverse of the tracheary elements. The network of phloic elements should be well developed in the photosynthetic regions of the leaves and stem gradually decreasing in number toward the rooted basal portion. The xylary system in Monotropa is well developed for the uptake of water and mineral nutrients through the roots as affirmed by Gleeson (1970). Henderson (1919) maintained that there was an elaborate system of phloem tissue extending throughout the entire axial distance from the roots to the leaf-scales. Gleeson (1970) negated this by describing the phloic distribution as sparse in the root and stem regions. The results of the present study advances the notion of a decrease in the number of sieve elements with increased distance from the roots, culminating in the total loss of sieve elements from the leaf-scales. The heterotrophic Monotropa would not require an extended phloem, especially in the leaf-scale and stem regions where photosynthesis no longer occurs.

The present report on sieve element distribution supports the unidirectional function of the vascularization of Monotropa uniflora L. Energy substrates as well as water and minerals may be taken in and translocated from the basal portions of the plant to the area of reproduction in the developing apical flower. Gleeson's (1970) work and the results of the present study also show that a substance collects in the middle and upper tissues of the aerial stem. Gleeson (1970) refers to this material as insoluble polysaccharides and it is thought to be the stored energy substrate required for the proper development of the flower.

Major clues to the evolutionary condition of Monotropa may be discovered by the study of the vascular tissue component cells. The evolutionary trends in angiosperm sieve element specialization are listed by Fahn (1974): (1) there is a gradual localization of highly specialized sieve areas on the end walls; (2) the end walls become more transverse than oblique in orientation; (3) sieve plates change from compound to simple; (4) and there is a gradual reduction of the lateral wall sieve areas. Plants containing such specialized sieve elements are considered to be more advanced (Fahn, 1974). Monotropa uniflora L. sieve elements exhibit specialized sieve areas (sieve plates) located on the transverse end walls. The lateral sieve areas vary in number of pores from one to seven and are reduced in the actual number of lateral wall locations. The advanced status of Monotropa uniflora L. can be argued from these observations.

The loss of the photosynthetic activity may have had a striking effect on the individual sieve element structure. Esau (1969) asserts that the occurrence of larger pores on the sieve plates with respect to smaller pores found on the lateral walls is an indication of evolutionary advancement.

The tendency to have larger sieve plate pores is common among angiosperms, while the gymnosperms and lower vascular plants generally possess sieve pores that are smaller and more uniform in size ranging from a fraction of a micron to 1-2 microns (Esau, 1969). *Monotropa* sieve pore size in terms of callose plug diameters was found in the present study to range between 0.55 and 1.1 microns. The pores on the sieve plate appear to be in the same size range as the pores on the lateral walls. The similarity in the pore sizes is one correlation that can be made between sieve elements found in *Monotropa uniflora* L. and sieve elements found in the lower vascular plants. Burr and Evert (1973) found that sieve pore sizes were the same on the end and lateral walls of *Selaginella braussiana*. The authors of that research felt that the common pore size is a widely distributed phenomenon in lower vascular plants.

Another striking feature of *Monotropa* phloem is the absence of conspicuous cells fitting the definition by Esau (1969) of companion cells. There is evidence for some sort of deliberate intercellular connection between a sieve element and a bundle parenchyma cell in *Monotropa*. This type of phenomenon is reported to occur with regular frequency between sieve elements and adjacent parenchyma cells of the lower vascular plants. Electron microscopic investigations conducted by Warmbrodt and Evert (1974), indicated that the intercellular connections found in *Lycopodium lucidulum* consisted of a plasmadesma on the parenchyma side and a lateral wall pore on the sieve element side. This may also be the situation in *Monotropa uniflora* L.

Still another correlation between the lower vascular plants and *Monotropa* is the apparent absence of sieve element P-protein as Esau (1969) described the substance. A survey of the sieve elements found in *Selaginella* (Burr and Evert, 1973), certain polypodiaceous ferns (Evert and Eichhorn, 1974), *Isoetes* (Kruatrachue and Evert, 1974), and *Lycopodium* (Warmbrodt and Evert, 1974) failed to show the occurrence of P-protein. In some of the lower vascular plants studied, however, there were various substances found to resemble P-protein (Evert and Eichhorn, 1974). On the light microscopic level there was no indication of any P-protein-like material in the sieve tube members of *Monotropa uniflora* L.

The results of this light microscopic study on *Monotropa* suggests a probable ancient photosynthetic ancestor. The sieve element cytology indicates a return to a less specialized sieve element approaching those found in lower vascular plants. This could be considered an advancement. The trend toward the less specialized condition and the apparent total loss of sieve elements in the leaf-scales are probably the major effects brought about by the loss of the photosynthetic pathways.

The only way to resolve discrepancies and present a clear picture of sieve elements cytology is through electron microscopy. By studying electron photomicrographs the true nature of the sieve pore can be realized. It will be important in such studies to make measurements of the pores, determine their contents, and characterize the intercellular connections between sieve elements and parenchyma cells. Evolutionary questions raised about <u>Monotropa</u> <u>uniflora</u> L. may yet be answered.

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FIG. 1. *Monotropa uniflora* L. habit photograph.

①

FIG. 2. Transection through a stem just below the flower showing immature vascular bundles arranged in a eustele. X116.9. Details: E, epidermis; C, cortex; P, pith; X, xylem cell; S, phloem cell; A, parenchyma cell.

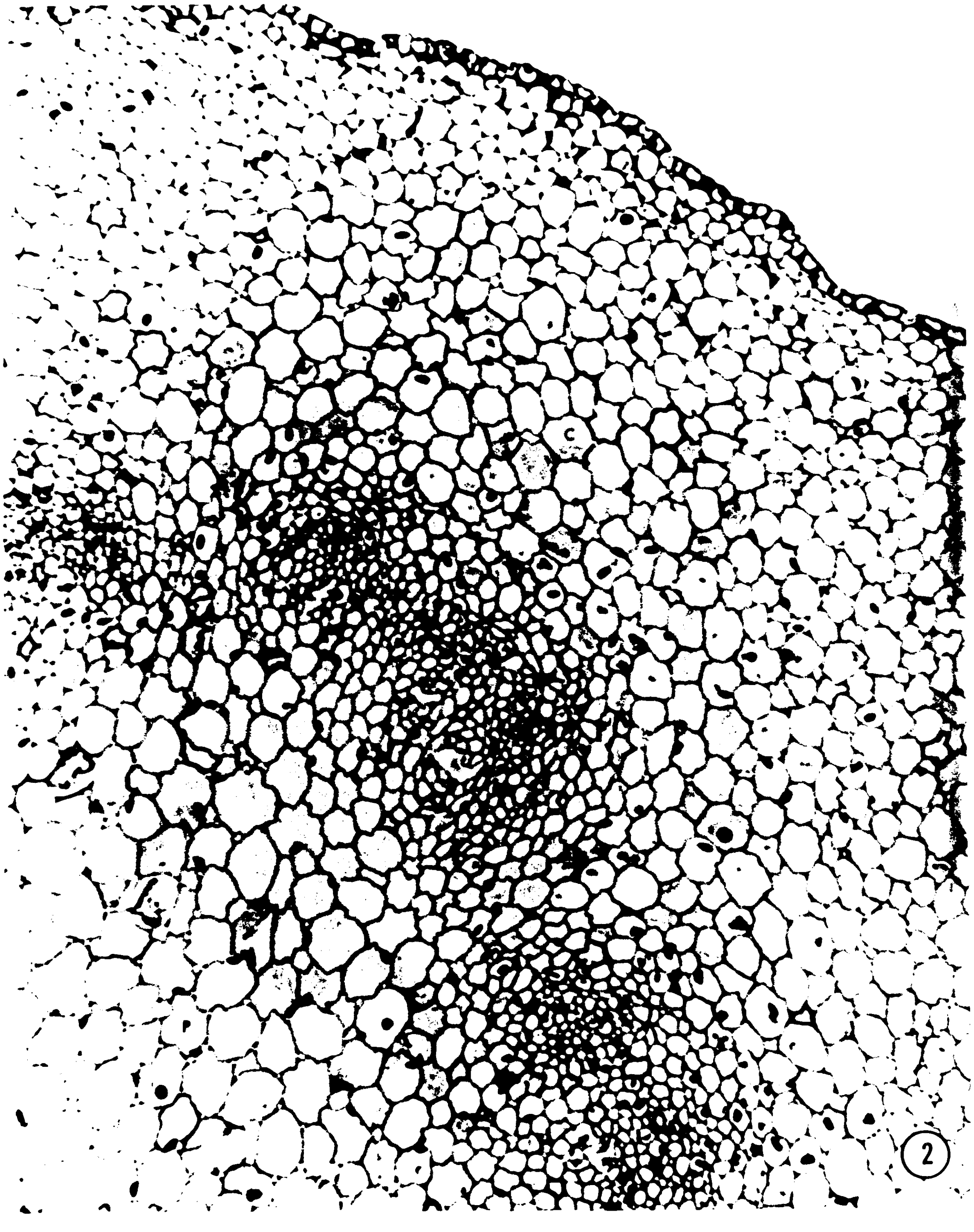

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FIG. 3. Transection through mature stem near the base of the plant. A lignified pith and well developed vascular bundles are evident. X116.9.

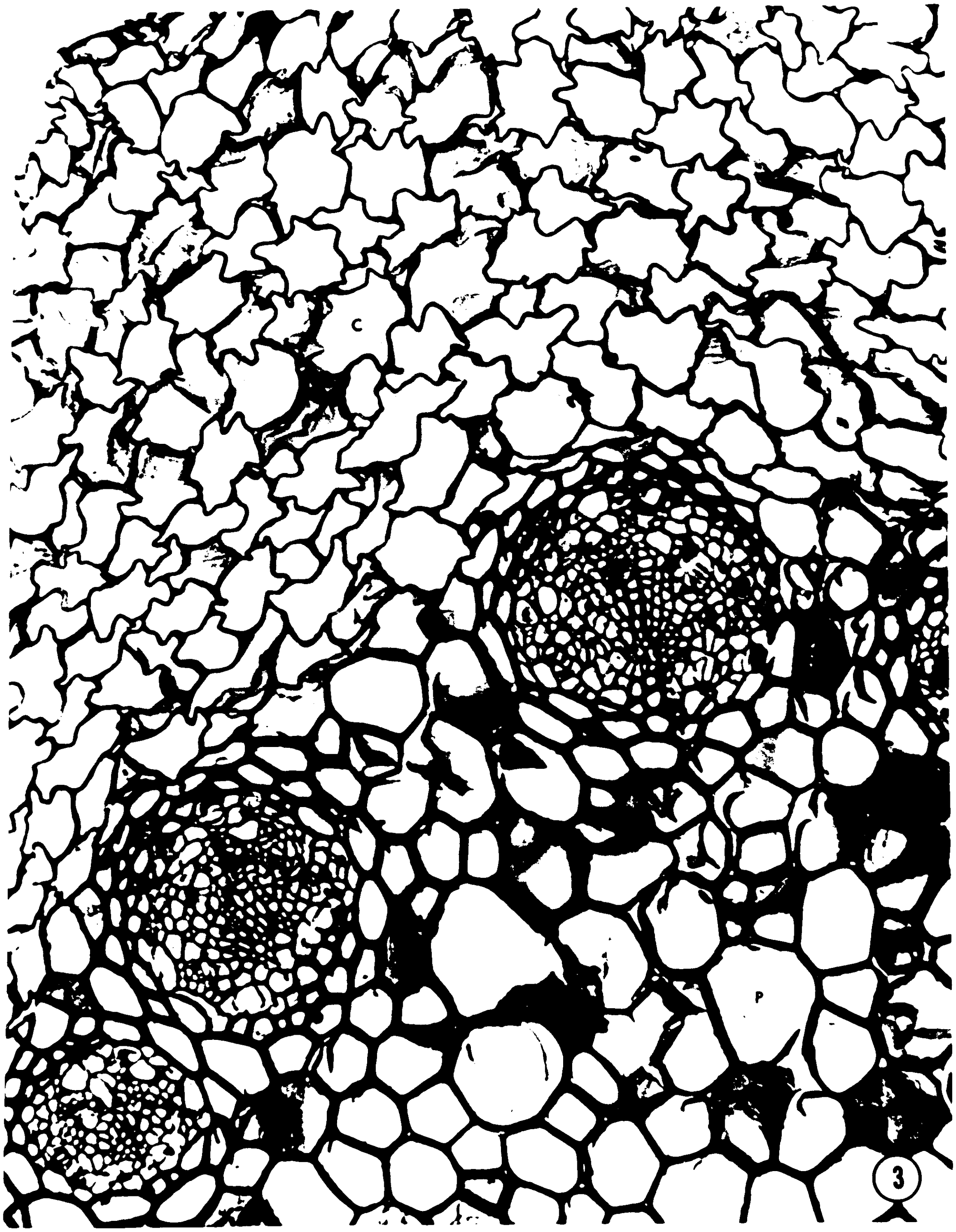

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FIG. 4-8. --FIG. 4. Longitudinal section showing storage material in stem epidermal and cortical cells. X182.9. --FIG. 5. Longitudinal section showing immature insect form in cortex. X500. --FIG. 6. Transection through stem cortex showing insect in cross section. X182.9. --FIG. 7-8. Face view of stem epidermal stomata showing different guard cell arrangements. X500.

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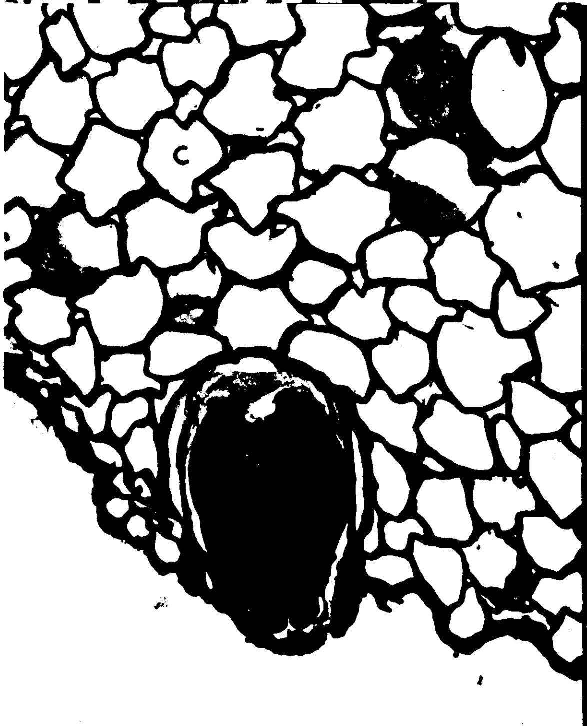

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FIG. 9-10. --FIG. 9. Transection through stem showing lignified parenchyma cells delimiting each vascular bundle. Storage material in these parenchyma cells is evident. X182.9. --FIG. 10. Longitudinal section through stem vascular tissue showing tracheary elements indicated by the arrow. X182.9.

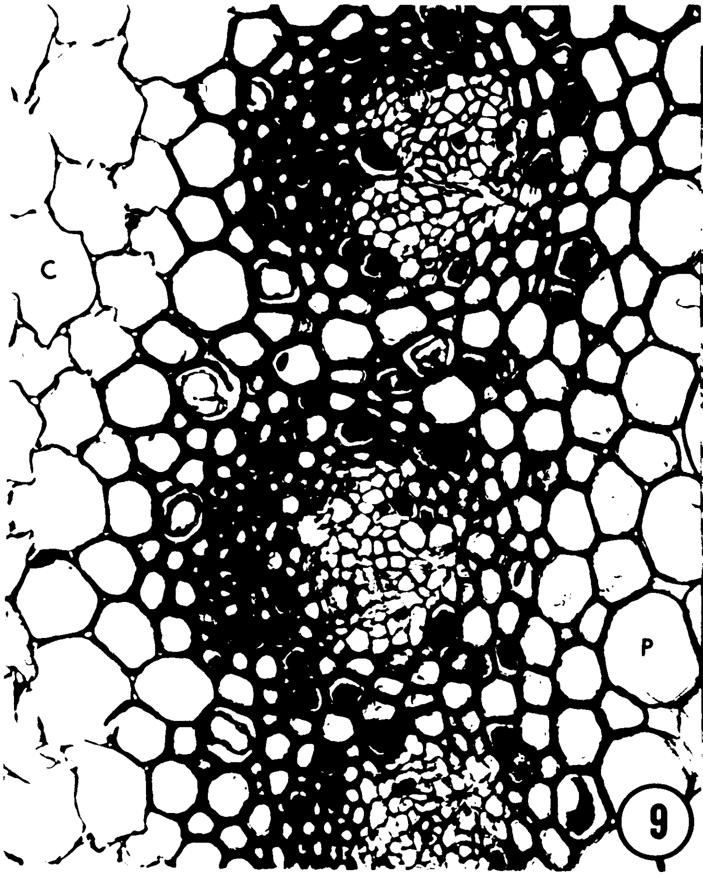

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FIG. 11-16. --FIG. 11. Transection through vascular bundle showing parenchyma cells and sieve tube members. Arrow indicates sieve plate exhibiting callose plugs. X1212. --FIG. 12. Transection through vascular bundle showing tracheary elements, sieve tube members, and parenchyma cells. Arrow indicates callose deposition between sieve tube member and nucleated parenchyma cell. X1212. --FIG. 13. Longitudinal section showing sieve plates with arrow indicating callose plugs. X1212. --FIG. 14. Longitudinal view showing sieve plate between two sieve tube members. X1212. --FIG. 15. Longitudinal view of sieve tube member with arrow indicating lateral sieve area. X1212. --FIG. 16. Longitudinal view of vascular tissue with the arrow indicating a possible connection between sieve tube member and a nucleated parenchyma cell. X1212.

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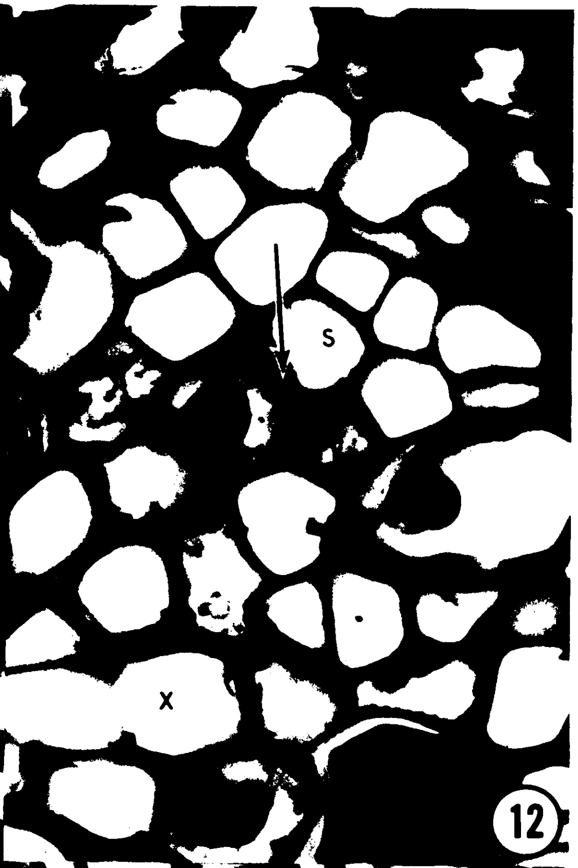

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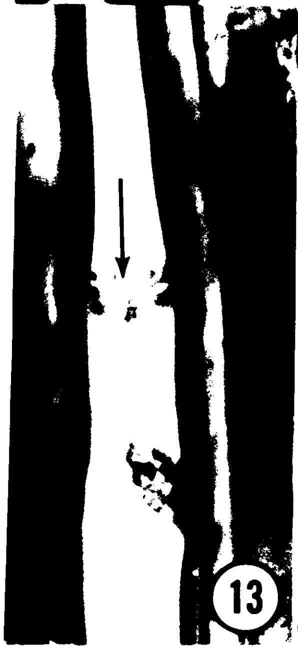

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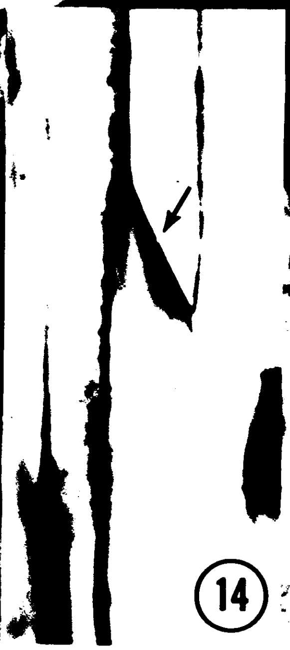

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FIG. 17-20. --FIG. 17. Transection through leaf-scale with arrow indicating a vein. Undifferentiated mesophyll is evident. X182.9. --FIG. 18. Transection through leaf-scale major vein with arrows indicating tracheary elements surrounded by nucleated parenchyma. X500. --FIG. 19. Transection through a minor vein with arrow pointing to tracheary element. Nucleated parenchyma cells surround the xylem. X500. --FIG. 20. Transection through stem showing leaf-scale trace leaving the bundle formation. X182.9.

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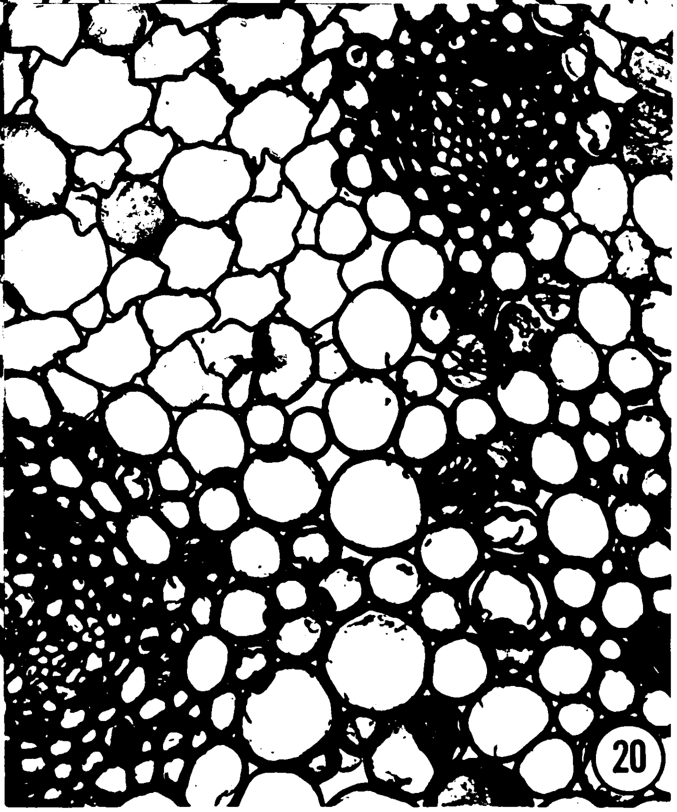

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FIG. 21. Drawing showing longitudinal section through two sieve tube members. Callose plugs in sieve plate pores.

FIGURE 21

Sieve Pore Callose Plugs